Graphical abstract

Peripheral site and acyl pocket define selective inhibition of mouse butyrylcholinesterase by two biscarbamates **pp xxx–xxx**

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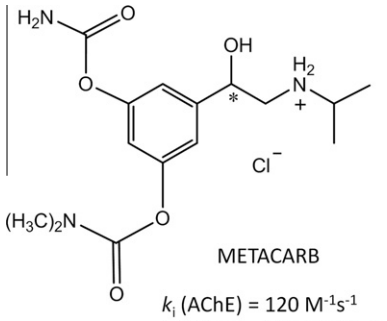

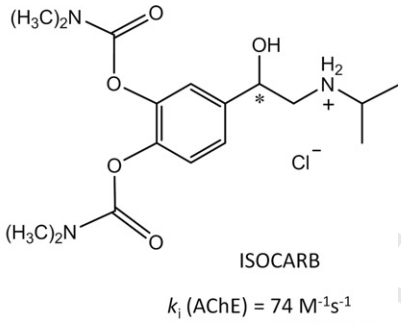

Highlights

▶ Biscarbamates metacarb and isocarb are selective butyrylcholinesterase inhibitors. ▶ Inhibition selectivity of metacarb and isocarb is lower than that of bambuterol. ▶ Carbamylation rate is affected by disposition of carbamic groups on benzene ring. ▶ Selective inhibition of BChE is determined mainly by BChE residues Q119 and L286.

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Peripheral site and acyl pocket define selective inhibition of mouse butyrylcholinesterase by two biscarbamates

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ABSTRACT

In this study we related metacarb (*N*-(2-(3,5-bis(dimethylcarbamoyloxy)phenyl)-2-hydroxyethyl)propan-2-aminium chloride) and isocarb (*N*-(2-(3,4-bis(dimethylcarbamoyloxy)phenyl)-2-hydroxyethyl)propan-2-aminium chloride) inhibition selectivity, as well as stereoselectivity of mouse acetylcholinesterase (AChE; 3.1.1.7) and butyrylcholinesterase (BChE; 3.1.1.8) to the active site residues by studying the progressive inhibition of AChE, BChE and six AChE mutants with racemic and (*R*)-enantiomers of metacarb and isocarb. Metacarb and isocarb proved to be very potent BChE inhibitors with inhibition rate constants in the range of 10^3–10^4 M^{-1} s^{-1}. For metacarb and isocarb, inhibition of BChE w.t. was 260 and 35 times, respectively, faster than inhibition of AChE w.t. For four mutants inhibition was faster than for AChE w.t. but none reached the inhibition rate of BChE. The highest increase in the inhibition rate (about 30 times for metacarb and 13 times for isocarb) was achieved with mutants F295L/Y337A and Y124Q meaning that selective inhibition of mouse BChE is dictated mainly by two amino acids from BChE: leucine 286 from the acyl pocket and glutamine 119 from the peripheral site. Wild type enzymes displayed pronounced stereoselectivity for (*R*)-enantiomers of metacarb and isocarb. Interestingly, the residues that define selective inhibition of mouse BChE by biscarbamates also affect the stereoselectivity of enzymes.

Introduction

Hydrolysis of neurotransmitter acetylcholine is a physiological role of acetylcholinesterase (AChE; EC 3.1.1.7)[3] that is irreplaceable in maintaining optimum neurotransmission. Butyrylcholinesterase (BChE, EC 3.1.1.8) is closely related to AChE, but far less specific and capable of hydrolysing a broad spectrum of structurally different substrates including acetylcholine. In animals and humans, BChE acts as a scavenger of many natural and synthetic anticholinesterase compounds before they can reach AChE, and it is involved in the metabolism of various xenobiotics (cocaine, heroin) [1,2].

AChE and BChE share more than 50% of identical amino acid sequence and almost the same backbone structure [3]. The crystal structure of both cholinesterases showed that their active site is located in the 20 Å deep gorge [4,5]. Kinetic and structural studies revealed the existence of four functional sub-domains within the gorge: the catalytic triad accompanied by the "oxyanion hole", acyl pocket and choline binding site located at the bottom of the gorge, and the peripheral site located at the entrance of the gorge [6]. AChE's active site is lined with 14 aromatic amino acid residues, six of which are replaced with aliphatic residues in BChE (Fig. 1), which [7] results in different reactivity toward substrates and other covalent modifiers (acylating, carbamylating or phosphylating agents) as well as toward noncovalent ligands [8–11].

Carbamates react with AChE and BChE by forming the covalent bond between the carbamic group of a carbamate and the hydroxyl group of catalytic serine. Due to a slow decarbamylation rate, carbamates are progressive inhibitors of cholinesterases, a feature that has been successfully used in the treatment of symptoms of neurodegenerative disorders related with cholinergic neurotransmission where inhibition of AChE represents initial and primary treatment [3]. A growing number of studies pointing to the role of BChE in the regulation of normal neuronal function resulted in the idea of considering BChE as a therapeutic target too [12,13]. As a BChE-selective carbamate, bisnorcymserine is currently pursued for clinical development as an Alzheimer therapeutic [14]. Besides selectivity, when interacting with chiral compounds, cholinesterases are also stereoselective enzymes discriminating

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[3] *Abbreviations used:* AChE, acetylcholinesterase; BChE, butyrylcholinesterase; ATCh, acetylthiocholine iodide; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid).

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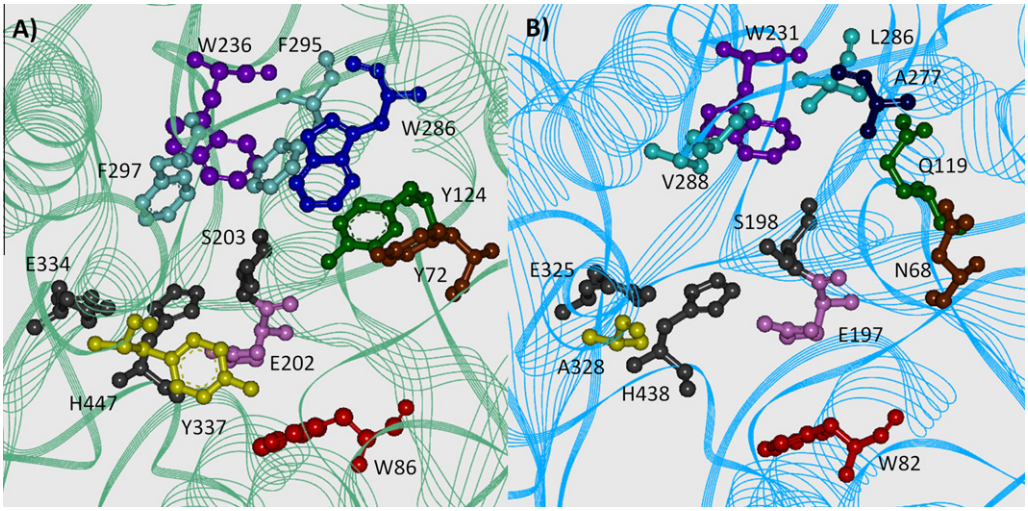

Fig. 1. View of the active site of: panel A) AChE (PDB code 2HA3) and panel B) BChE (PDB code 2PM8). Amino acid residues conserved in AChE and BChE are highlighted: catalytic triad S203, H447 and E334 (gray), E202 (pink), W86 (red) and W236 (violet). The rest of amino acids from AChE, panel A, are those mutated to mimic BChE active site: Y337 (yellow) from the choline binding site, F295 and F297 (turquoise) from the acyl pocket and Y72 (brown), Y124 (green) and W286 (blue) from the peripheral site. Instead of these, A, L, V, N, Q and A are in the active site of human BChE, respectively. Instead of A and R at positions 277 and 288 in human BChE, in mouse BChE R and I are at these positions. Numbers next to the amino acid on the panel A refer to the position of the amino acid in mouse AChE, and on the panel B to the position in human BChE.(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

enantiomers of organophosphorous and quinuclidinium esters, reversible inhibitors, and carbamates [8,15–17]. It was reported previously that AChE preferred the (−)-enantiomers of physostigmine, cymserine and physovenine, while (*R*)-bambuterol was preferred by both, AChE and BChE [18,19].

In the present study, we have analysed the interaction of metacarb (*N*-(2-(3,5-bis(dimethylcarbamoyloxy)phenyl)-2-hydroxyethyl)propan-2-aminium chloride) and isocarb (*N*-(2-(3,4-bis-(dimethylcarbamoyloxy)phenyl)-2-hydroxyethyl)propan-2-aminium chloride) in the reaction with mouse AChE w.t., BChE w.t. and six AChE mutants that correspond to residues in mouse BChE (Fig. 1). Metacarb and isocarb are structural analogues of bambuterol, one of the most potent (inhibition rate constant in 10^4 M^{-1} s^{-1} range) and the most selective BChE inhibitors that inhibits human and mouse BChE about 20000 and 16000 times faster than AChE, respectively [20–22]. Metacarb, isocarb and bambuterol differ in the size of the alcohol part of ester or/and in the disposition of carbamic groups on the benzene ring (Fig. 2). Our previous study has shown that metacarb and isocarb are selective inhibitors of human BChE compared to human AChE, but less compared to bambuterol [9]. In the reaction with metacarb, isocarb and bambuterol as chiral molecules with stereogenic centre on the alcohol part of the molecule, cholinesterases are stereoselective with preference to (*R*)-enantiomers of those biscarbamates as was demonstrated previously [9,17,18,23]. The aim of the work was to assign the inhibition potency and selectivity of metacarb and isocarb, as well as stereoselectivity of cholinesterases, to amino acids of the active site gorge.

Materials and methods

Chemicals

Enzyme inhibitors, racemic and (*R*)-enantiomers of metacarb [*N*-(2-(3,5-bis(dimethylcarbamoyloxy)phenyl)-2-hydroxyethyl)-propan-2-aminium chloride] and isocarb [*N*-(2-(3,4-bis(dimethylcarbamoyloxy)phenyl)-2-hydroxyethyl)propan-2-aminium chloride] (Fig. 2), were synthesised as described previously [9]. Enantiomeric purity was 99.5% for (*R*)-metacarb and 83.1% for (*R*)-isocarb, determined by HPLC on chiral columns Chiralpak-AD and Chiralcel-OD, respectively. Enzyme substrate acetylthiocholine iodide (ATCh) and thiol reagent 5,5́-dithiobis(2-nitrobenzoic acid) (DTNB) were purchased from Sigma Chemical Co., USA. All other reagents were of analytical grade.

Enzymes

Mouse AChE w.t., BChE w.t. and six AChE mutants were recombinant enzymes prepared at the University of California at San Diego, USA, as described previously [16,24]. The mutated residues were in the choline binding site (Y337A), in combination with the acyl pocket residues (F295L/Y337A, F297I/Y337A, F295L/F297I/

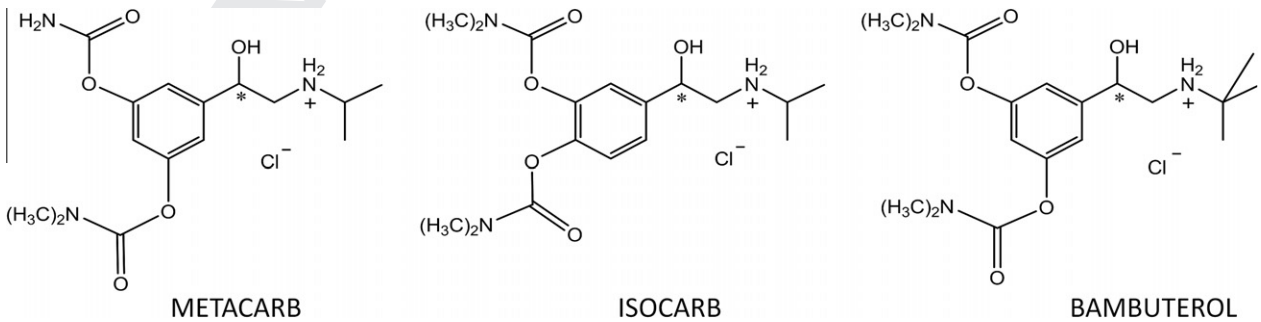

Fig. 2. Structures of metacarb, isocarb and bambuterol.

Y337A) and at the peripheral site (Y124Q, Y72N/Y124Q/W286R), and correspond to residues of BChE (Fig. 1). Unless otherwise stated, throughout the paper, numbers next to the amino acids refer to the numbering of amino acid residues in mouse AChE.

Enzyme activity measurements

The activity of enzymes was measured with 1.0 mM ATCh in 0.1 M phosphate buffer, pH 7.4. using modified Ellman spectrometric method [25]. Measurements were caried out at 25 °C and 412 nm using Cary 300 Varian Inc. (Australia) spectrophotometer.

Enzyme inibition

Enzyme samples were incubated for up to 30 min with the carbamates. The inhibition reaction was stopped by adding ATCh (1.0 mM final concentration), and the extent of inhibition was determined by measuring the residual activity. At least three different concentrations of carbamates, in the range of 0.05–2000 μM, were used in at least two experiments.

Kinetic constants k_i (the overall inhibition rate constant), K_i (the enzyme-carbamate dissociation constant) and k_{max} (the maximal first-order inhibition rate constant) describing the progressive (time-dependent) inhibition by carbamates were determined as described previously [17]. The overall inhibition rate constant (k_i) represents a first step in the metacarb and isocarb hydrolysis by cholinesterases. The determination of kinetic constants was carried out using GraphPad Prism program.

Contribution of mutations to the transition state energy

The difference in Gibbs energy between the transition state and the ground state is the Gibbs energy of activation ($\Delta G^{\ddagger}$) and it is calculated from the association rate constant, applying the transition state theory [26]. The change in the transition state energy caused by mutations, $\Delta\Delta G^{\ddagger}_{mut\text{-}wt}$, was calculated according to the equation applied for the overall catalytic rate constants of enzyme substrate reactions [26]:

$$\Delta\Delta G^{\ddagger}_{mut\text{-}wt} = -RT\ln(k_{i(mut)}/k_{i(w.t.)})$$

where $k_{i(w.t.)}$ and $k_{i(mut)}$ are the second-order rate constants of AChE w.t. and of the mutant for the inhibition by metacarb or isocarb. R equals 8.314 JK^{-1} mol^{-1} and T equals 298 K. $\Delta G^{\ddagger}$ of AChE was taken as a referent value.

Results and discussion

Biscarbamates, metacarb and isocarb, progressively inhibited all tested cholinesterases and the inhibition followed first-order kinetics at any given inhibitor concentration (Figs. 3A and 4A). For all studied cholinesterases, except for double and triple mutants with F297I mutation, the first-order rate constants $k_{[AB]}$ were a linear function of metacarb and isocarb concentrations (Fig. 3B) wherefrom the overall inhibition rate constants (k_i) were determined (Table 1). For mutants F297I/Y337A and F295L/F297I/Y337A, the relation between $k_{[AB]}$ constants and concentrations of metacarb and isocarb displayed hyperbolic shape (Fig. 4B). In these cases maximal inhibition rate constants (k_{max}) and enzyme-carbamate dissociation constants (K_i) were determined and the overall inhibition rate constants (k_i) were calculated from the ratio $k_i = k_{max}/K_i$ (Table 2). Mutations studied in this paper caused a change in the transition state energy relative to the transition state energy of AChE w.t., and the effect is expressed as a change in Gibb's energy, $\Delta\Delta G^{\ddagger}_{mut\text{-}wt}$, between mutant and AChE w.t. (Table 1). Negative $\Delta\Delta G^{\ddagger}_{mut\text{-}wt}$ value indicates a more energetically favourable reaction of mutants relative to the AChE w.t.

Inhibition selectivity

Metacarb and isocarb were found to be very fast BChE inhibitors having overall inhibition rate constants in the range of 10^3–10^4 M^{-1} s^{-1} order of magnitude (Table 1), similar to those previously determined for human BChE variants [9]. Both carbamates proved to be selective inhibitors of BChE inhibiting BChE w.t. 260 and 35 times faster than AChE w.t., respectively (Table 1). About 7 times lower inhibition selectivity of isocarb compared to metacarb is primarily the result of slowing down the isocarb inhibition rate of BChE w.t. The rate of carbamylation is determined by accommodation of the ligand into the active site of enzyme, which includes positioning carbamic group into the oxyanion hole. Therefore, lower selectivity of isocarb could be assigned to ortho-disposition of carbamic groups on the benzene ring (Fig. 2).

Compared to bambuterol's pronounced selectivity [17], the inhibition selectivity of metacarb and isocarb decreased 30- and 240-fold, respectively, which could be explained by different sizes of the alcohol part of carbamates (*tert*-butyl group on bambuterol *vs. iso*-propyl group on metacarb), or in case of isocarb *vs.* bambuterol also by different dispositions of carbamic groups on the benzene ring. The decreased inhibition selectivity of metacarb and isocarb, compared to bambuterol, is also explained by about 10 times faster inhibition of AChE by these carbamates.

Effect of mutations on inhibition rate and selectivity

It was demonstrated previously that bambuterol inhibition selectivity toward BChE is dictated largely by residues alanine 328 from the choline binding site (337 by mAChE numbering) and glutamine 119 from the peripheral site (124 by mAChE numbering) [21]. The effect of mutations of residues lining the active site gorge of AChE (Fig. 1) on the inhibition rate and inhibition selectivity of metacarb and isocarb for BChE was observed as a ratio of the overall inhibition rate constant of mutants and AChE w.t. (Table 1). The residues in three distinct domains of the active site of AChE were mutated to mimic the residues on the corresponding places in BChE (Fig. 1). Mutants were selected on the basis of previous studies with bambuterol [17,21].

Inhibition by metacarb and isocarb was faster than for AChE w.t. for all mutants except double and triple mutants with F297I mutation. The highest increase in the inhibition rate by metacarb (29 times) was achieved by mutants F295L/Y337A and Y124Q, while the fastest inhibition by isocarb was accomplished by F295L/Y337A (13 times). Inhibition of these mutants by isocarb was only three times and inhibition by metacarb about 9 times slower than that of BChE w.t.

The increase in the inhibition rate by mutant F295L/Y337A for both carbamates, compared to AChE w.t., could indicate that binding of biscarbamates metacarb and isocarb into the AChE active site is stabilised with π–π interactions between carbamate aromatic ring and the phenylalanine from the acyl pocket and the aromatic ring of tyrosine from the choline binding site. But these interactions seem either to be strong enough to prevent proper orientation of carbamates inside the AChE active site for the reaction with catalytic serine, or to be stabilising the Michaelis type of complex between biscarbamate and AChE (however, both resulting in the slow carbamylation process). The fact in favour of the latter assumption is the non-linear relationship between $k_{[AB]}$ and concentration of biscarbamate for AChE (Fig. 3B). Mutations Y337A and F295L lead to the enlargement of the choline binding site and the acyl pocket, and to the elimination of π–π interactions that are present during carbamylation of AChE w.t. Mutation Y337A de-

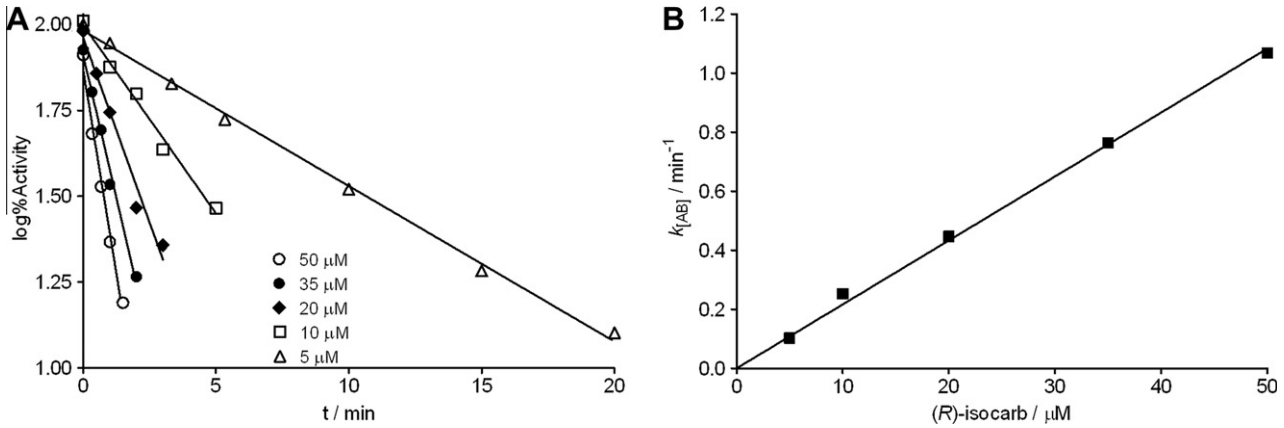

Fig. 3. Inhibition of Y72N/Y124Q/W286R by racemic isocarb. Points on panel A indicate the logarithm of residual activity *vs*. time of inhibition. The slopes of the line, the first-order rate constants $k_{[AB]}$, were plotted as a function of inhibitor concentration (panel B), wherefrom the overall inhibition rate constant (k_i) was calculated.

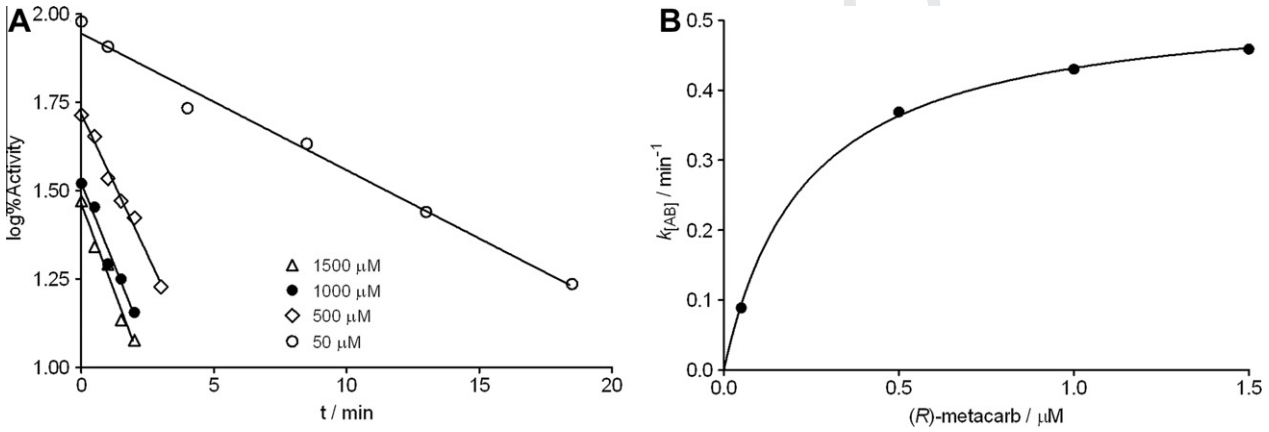

Fig. 4. Inhibition of F297I/Y337A by (*R*)-metacarb. The points on panel A indicate the logarithm of residual activity *vs*. time of inhibition. The slopes of the line, the first-order rate constants $k_{[AB]}$, were plotted as a function of inhibitor concentration (panel B), wherefrom the maximal inhibition rate constant (k_{max}) and the enzyme-carbamate dissociation constant (K_i) were calculated. The overall inhibition rate constant, k_i, was calculated from the ratio $k_i = k_{max}/K_i$.

Table 1
Inhibition of AChE w.t., BChE w.t. and six AChE mutants by racemic and (*R*)-enantiomers of metacarb and isocarb.

Enzyme	Metacarb					
	Racemate			(*R*)-		
	k_i/M^{-1} s^{-1}	$k_i/k_{i(AChE\ w.t.)}$	$\Delta\Delta G^{\ddagger}_{mut\text{-}wt}$/kJ mol^{-1}	k_i/M^{-1} s^{-1}	$k_i/k_{i(AChEw.t.)}$	$k_{i(R)}/k_{i(rac)}$
AChE w.t.	120 ± 5	1	0	190 ± 5	1	1.6
BChE w.t.	31000 ± 800	280	−14	57000 ± 2200	310	1.8
Y337A	260 ± 13	2.3	−2.0	420 ± 22	2.3	1.6
F295L/Y337A	3300 ± 110	29	−8.3	5500 ± 220	30	1.7
F297I/Y337A	22 ± 7	0.19	4.2	31 ± 14	0.17	1.4
F295L/F297I/Y337A	25 ± 9	0.22	3.9	21 ± 7	0.11	0.84
Y124Q	3600 ± 66	30	−8.4	5800 ± 110	32	1.6
Y72N/Y124Q/W286R	1500 ± 81	13	−6.4	2300 ± 95	13	1.5
	Isocarb					
AChE w.t.	74 ± 2	1	0	99 ± 2	1	1.3
BChE w.t.	2600 ± 87	36	−8.8	4600 ± 1100	47	1.8
Y337A	160 ± 7	2.1	−2.0	140 ± 7	1.4	0.88
F295L/Y337A	990 ± 24	13	−6.3	2300 ± 54	23	2.3
F297I/Y337A	64 ± 23	0.89	0.37	40 ± 17	0.40	0.63
F295L/F297I/Y337A	30 ± 6	0.41	2.2	31 ± 20	0.32	1.0
Y124Q	600 ± 11	8.2	−5.2	1100 ± 44	11	1.8
Y72N/Y124Q/W286R	300 ± 15	4.1	−3.5	370 ± 7	3.7	1.2

creased the transition state energy by 2.0 kJ mol^{-1} for both biscarbamates in comparison with AChE w.t. Assuming a cumulative effect of mutations F295L and Y337A, it seems that an additional decrease in the transition state energy of 6.3 kJ mol^{-1} for metacarb and of 4.3 kJ mol^{-1} for isocarb, achieved by F295L/Y337A, is due to the effect of mutation F295L. Based on this assumption we may

Table 2
Dissociation constants for Michaelis-type of enzyme-carbamate complex (K_i) and maximal first-order rate constants (k_{max}) for F297I/Y337A and F295L/F297I/Y337A by racemic and (*R*)-metacarb, and racemic and (*R*)-isocarb.

Enzyme	Metacarb			
	Racemate		(*R*)-metacarb	
	k_{max}/s^{-1}	K_i/mM	k_{max}/s^{-1}	K_i/mM
F297I/Y337A	0.0069 ± 0.0009	0.31 ± 0.09	0.011 ± 0.002	0.34 ± 0.15
F295L/F297I/Y337A	0.023 ± 0.004	0.93 ± 0.30	0.034 ± 0.006	1.7 ± 0.5
	Isocarb			
	Racemate		(*R*)-isocarb	
F297I/Y337A	0.0022 ± 0.0002	0.034 ± 0.012	0.0029 ± 0.0004	0.073 ± 0.028
F295L/F297I/Y337A	0.015 ± 0.001	0.49 ± 0.09	0.019 ± 0.006	0.60 ± 0.34

Table 3
Relation between the overall inhibition rate constants (k_i) for BChE w.t., AChE w.t. and six AChE w.t. mutants by racemic and (*R*)-enantiomer of metacarb, isocarb and bambuterol. Stereoselectivity of bambuterol determined from the ratio $k_{i(R)}/k_{i(S)}$ is displayed within the brackets [17].

	$k_{i(R)}/k_{i(rac)}$		
	Metacarb	Isocarb	Bambuterol
AChE w.t.	1.6	1.3	1.2 (3.7)
BChE w.t.	1.8	1.8	1.1 (4.2)
Y337A	1.6	0.88	1.1 (15)
F295L/Y337A	1.7	2.3	1.4 (15)
F297I/Y337A	1.4	0.63	1.7 (12)
F295L/F297I/Y337A	0.84	1.0	1.5 (1.8)
Y124Q	1.6	1.8	0.64 (1.6)
Y72N/Y124Q/W286R	1.5	1.2	1.2 (1.4)

conclude that mutation F295L causes about 15 times faster inhibition by metacarb and about 7 times by isocarb, compared to AChE w.t.

Mutation of the second phenylalanine from the AChE acyl pocket, F297I, slows down the inhibition rate compared to AChE w.t. and leads to an increase in the transition state energy of F297I/Y337A and F295L/F297I/Y337A for both carbamates. Moreover, the accumulation of the Michaelis type complex of enzyme-carbamate was observed (Fig. 4A) for F297I/Y337A and F295L/F297I/Y337A, allowing k_{max} and K_i constants (Table 2) to be determined. Although k_i constants for the inhibition by metacarb were similar for F297I/Y337A and F295L/F297I/Y337A, F297I/Y337A had three times higher affinity ($1/K_i$) for metacarb than F295L/F297I/Y337A. Slower inhibition rate of mutants with mutation F297I was also observed in the inhibition of cholinesterases by bambuterol [17]. Similar results were obtained with cholinesterases inhibited by physostigmine and phenserine where it was shown that mutation F297I caused a decrease and F295A an increase in the inhibition rate compared to AChE w.t. [18].

Peripheral site mutations, Y124Q and Y72N/Y124Q/W286R, increased the volume of the AChE active site gorge allowing the studied biscarbamates to access easily the gorge and catalytic serine. Inhibition of Y124Q mutant was about two times faster than Y72N/Y124Q/W286R for both biscarbamates. Multiple mutations in the peripheral site (Y72N/Y124Q/W286R) diminished the effect of mutation Y124Q meaning that Y72 and W286 did not contribute to the formation of favourable interactions for successful inhibition by metacarb and isocarb. Compared to the impact of mutation F295L on the inhibition rate of mutant F295L/Y337A, it seems that the inhibition selectivity of metacarb to BChE is dictated mainly by residue Q124 (Q119 in mouse BChE), while the inhibition selectivity of isocarb is equally affected by L295 and Q124 (L286 and Q119 in mouse BChE, respectively).

Carbamylation stereoselectivity

Metacarb and isocarb are chiral molecules with a stereogenic centre on the alcohol part of the molecule (Fig. 2), and stereoselectivity of cholinesterases could be expected. Due to difficulties in the synthesis of (*S*)-enantiomers of metacarb and isocarb, stereoselectivity of cholinesterases to metacarb and isocarb enantiomers could not be quantified, but was estimated from the ratio of the overall inhibition rate constants for racemate and (*R*)-enantiomer of metacarb and isocarb (Table 1), and compared with those for bambuterol [17]. Table 3 shows the ratio of the overall inhibition rate constants for cholinesterases by racemic and (*R*)-enantiomers of metacarb, isocarb and bambuterol, as well as bambuterol's determined stereoselectivity calculated from $k_{i(R)}/k_{i(S)}$ [17]. The comparison of $k_{i(R)}/k_{i(rac)}$ reveals *that* similar stereoselectivity to that of bambuterol enantiomers can be expected for most tested cholinestrases. Wild type cholinesterases, especially BChE, may display even higher stereoselectivity than the four times higher preference that was demonstrated earlier for (*R*)-bambuterol [17]. Higher stereoselectivity can be expected by F295L/Y337A and isocarb enantiomers (ratio of $k_{i(R)}/k_{i(rac)}$ was 2.3 *vs.* 1.4 for bambuterol), and in the case of Y124Q where the ratio was about 1.7 for metacarb and isocarb *vs.* 0.64 for bambuterol. The inversion of stereoselectivity could be expected for F297I/Y337A inhibited by isocarb. Despite the pronounced stereoselectivity of inhibition by bambuterol, inhibition rate constants of (*R*)-bambuterol were very close to that of racemate for most mutants, meaning that stereoselectivity of mutants depended mostly on slow inhibition rates by (*S*)-bambuterol. The same may probably stand for (*S*)-metacarb and (*S*)-isocarb.

Conclusion

In this paper we analysed the role of distinct functional sub-domains of mouse AChE active site in the reactivity toward metacarb and isocarb and in the selective inhibition of BChE by these biscarbamates, using six AChE mutants. Both biscarbamates proved to be selective BChE inhibitors compared to AChE, but far less than bambuterol. Metacarb and isocarb are structural analogues of bambuterol, a very powerful and very selective BChE inhibitor, and the observed reduction in inhibition selectivity is attributed to different dispositions of carbamic groups on the benzene ring of biscarbamates and to the size of the alcohol part of compounds. Inhibition selectivity of metacarb to BChE is dictated mainly by residue Q124 (Q119 in mouse BChE), while inhibition selectivity of isocarb is equally affected by L295 and Q124 (L286 and Q119 in mouse BChE, respectively). (*R*)-enantiomers of metacarb and isocarb inhibit AChE w.t. and BChE w.t. faster than the corresponding racemates, and when compared with the stereoselectivity of bambuterol enantiomers, it can be seen that stereoselectivity is dictated by the same residues as selectivity.

Conflict of interest statement

None.

Acknowledgments

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